MITOSIS AND AMITOSIS.

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It was once supposed that all forms of nuclear division were of the type which is now called amitosis in which the nucleus undergoes simple constriction. After the discovery of the complicated method of nuclear division now known as mitosis and the recognition of its very general occurrence in plants and animals, the doubt was expressed whether amitosis ever occurs as a normal process; its occurrence in pathological and degenerating cells is undoubted. However, the number of cases in which nuclear constriction is known to occur regularly in certain tissue cells is too great to warrant the belief that it is always a pathological phenomenon.

The only question of present importance in connection with this form of nuclear division is as to its bearing on the chromosome theory of heredity. If amitotic division of the nucleus, followed by division of the cell body, ever occurs in the germ cell cycle or in cleavage cells undergoing normal differentiation it would go far to disprove the "individuality" or persistent identity of chromosomes and consequently the chromosome theory of inheritance, since it is scarcely possible that individual chromosomes can be equally divided and their halves accurately distributed to daughter cells by the process of amitosis. The majority of cytologists at the present day concur in the opinion that amitosis does not normally occur in embryonic or germ cells and that when it does occur in such cells the ensuing development is abnormal. On the other hand some investigators maintain that amitosis occurs regularly and predominantly in the genesis of the germ cells and also in the blastomeres and embryonic cells of several species of animals and plants and for this reason among others they reject the chromosome theory. Child in particular has maintained this position in a series of papers (1907–1911) dealing primarily with the cestode *Monezia*,

but secondarily with several other animal forms ranging all the way from cœlenterates to birds.

Among other recent writers who have maintained a similar view are Hargitt (1904, 1911) on amitosis in the cleavage of the eggs of cœlenterates, Patterson (1908) in blastoderm cells of the pigeon, Glaser (1908) in embryonic cells of *Fasciolaria*, Jordan (1908) in spermatogonial divisions of *Aplopus*, Nathansohn (1900) in *Spirogyra*, Wasielewski (1902, 1903) in the root tips of *Vicia*, Gurwitsch (1905) in the blastomeres of *Triton*, Maximow (1908) in embryonic tissues of the rabbit, Jörgensen (1908) in the oögenesis of *Nephelis*, Moroff (1909) in the eggs of copepods, Knoche (1910) in the insect ovary, Nowikoff (1910) in bone and sinew cells of the young mouse, and Foot and Strobell (1911) in the ovary of *Protenor*—all of whom hold that amitosis may occur as a normal process in germinal and embryonic cells. Several of the authors named as well as R. Hertwig (1898), Lang (1901), Calkins (1901), Herbst (1909), Godlewski (1909) and Konopacki (1911) hold that there is no principal distinction between mitosis and amitosis and that they may both occur without interfering with normal processes of differentiation.

On the other hand, this view is contested by Boveri (1907) and Strasburger (1908) on general grounds and is not confirmed by the experiments of Häcker (1900) and Schiller (1909) on *Cyclops* eggs subjected to ether, nor by the experiments of Němec (1903) who repeated the work of Wasielewski on the root tips of *Vicia* subjected to chloral hydrate and reached the conclusion that the supposed amitoses are really modified mitoses. Richards (1909, 1911) and Harmon (1913) carefully and laboriously repeated Child's work on cestodes and found no evidence of amitosis in germ cells or cleavage cells, while on the other hand there was abundant evidence of mitosis. Child (1911) has reëxamined the question and reaffirms in the main his former opinion, and this has been confirmed by Young (1913), whose general conclusions, however, are so reactionary and even revolutionary that they cannot be accepted without further confirmation.

Boveri (1907) has said that if Child would establish his contention he must prove (1) that the binucleate condition which

he finds is actually due to a division, (2) that a portion of the cytoplasm is cut off around each of these nuclei, (3) that the cells arising in this manner again divide mitotically and have the normal number of chromosomes; in his opinion not one of these proofs has been furnished. The binucleate or multinucleate condition, he adds, may be due to an incomplete fusion of chromosomal vesicles after mitosis, as Rubaschkin (1905) has shown to be true for the blastomeres of *Triton*. Boveri concludes, therefore, that the individuality theory has not been shaken by this work on amitosis. There is no doubt that constricted or lobulated nuclei are sometimes found in germ cells or embryonic cells; the critical question is whether these are stages in the amitotic division of the nucleus and if so whether the cell bodies divide and are capable of normal development. It is evident from a large number of observations on a great variety of objects that constricted nuclei are not in themselves sufficient evidence that amitosis is occurring, for many such nuclei of living cells have been observed to lose the constriction and later to divide by mitosis. And even if such constricted nuclei actually divide, the chromosomal constitution of the cell could remain normal provided the cell body did not divide following the amitotic division of the nucleus.

As a matter of fact, amitotic division of the nucleus is rarely followed by division of the cell body. Macklin (1916) has recently studied amitosis in living tissue cells of the embryo chick and, although he was able to follow the process of nuclear division in successive stages in one and the same cell, in no case was such amitotic division of the nucleus followed by division of the cell body. Such amitotic division was sometimes followed by mitosis and in such cases the two or more nuclear vesicles in a cell gave rise to chromosomes which formed a single equatorial plate. Macklin concludes, therefore, that "there is nothing in nuclear amitosis opposed to the chromosome hypothesis"—with which opinion I entirely agree.

Unfortunately it is rarely possible to study all stages of nuclear and cell division in normal living cells, not only because of the indistinctness with which one sees what is going on inside the cell, but also because of the difficulty of keeping cells alive and

normal for a sufficient length of time under conditions of observation; furthermore, failure to observe amitosis in one hundred such cells would still leave it possible that it might occur in a second hundred. There is, however, a method by which the occurrence or non-occurrence of amitosis can be determined with great certainty and this is in the study of identical cell divisions in hundreds and thousands of individuals. This is possible only in the two maturation divisions which are so peculiar that they can always be distinguished from each other and from all other divisions, and in those early cleavages where the lineage of every cell is known and its method of division can be observed in thousands of different cases. If in every such dividing cell one sees mitosis only, it can be concluded that this is the invariable method of division in these cells. It is a significant fact, which Child himself admits, that in no such case have the nucleus and cell ever been seen to divide by amitosis, whereas in hundreds and thousands of cases they have been seen to divide by mitosis.

Nevertheless, in these very cells one frequently observes lobulated, constricted or bipartite nuclei which might be regarded as stages in amitosis were it not for the fact that the study of the lineage of these cells shows that they invariably divide by mitosis and that the peculiar shapes of the nuclei referred to are due to modifications of normal mitosis. Such constricted or bipartite nuclei occurring in cells the lineage of which was unknown would naturally be mistaken for cases of amitosis and it is very significant that the reports of amitosis in embryonic cells have been invariably in cells of unknown lineage.

This paper is based on a study of modified mitosis in the maturation and cleavage of the eggs of the marine gasteropod, *Crepidula plana*. A variety of nuclear forms are figured and described which resemble more or less closely stages of amitosis and yet it is perfectly certain that these nuclei divide only by mitosis, and the manner of origin of these pseudo-amitotic forms is plainly due to modifications of regular mitotic processes. These modifications were produced by subjecting dividing cells to abnormally high temperatures or to sea-water of abnormal densities, but other similar modifications are produced by many other abnormal conditions such as pressure, centrifugal force,

carbonic acid and various other chemical substances (Conklin, 1912). These different types of modified mitoses may be classified under the following heads: (1) Scattering of chromosomes and their failure to unite into a single nuclear vesicle, (2) amitotic or mitotic division of the nucleus without division of the cell body and the subsequent division of such binuclear or polynuclear cells, (3) separation of chromatin and achromatin and formation of cytasters, (4) persistence of nuclear membrane and formation of chromatic connections between daughter nuclei.

I. Scattering of Chromosomes and Their Failure to Unite Into a Single Nuclear Vesicle.

In the late anaphase of normal mitoses the chromosomes of the daughter plate stick together so that when the individual chromosomes begin to take in achromatic substance and to swell up into chromosomal vesicles the whole plate is converted into a mulberry-like mass which later becomes a single nuclear vesicle either by the fusion of the separate chromosomal vesicles or by their closer approximation. There is a growing body of evidence that in certain cases at least these closely appressed chromosomal vesicles do not completely fuse with one another but preserve their individuality (Bonnevie, 1908, for *Ascaris* and *Allium*; Vejdovsky, 1912, for *Ascaris* and *Decticus*; Wenrich, 1916, for *Phrynotettix*; Richards, 1917, for *Fundulus*). In other cases, when it is not possible to recognize a distinct vesicle for each and every chromosome, maternal and paternal chromosomes may form more or less distinct vesicles (Häcker, 1895, for *Cyclops*; Conklin, 1901, 1902, for *Crepidula*).

In certain abnormal conditions, and especially by means of temperatures higher than normal and by hypertonic sea-water, the division and separation of daughter chromosomes may be delayed or stopped and the chromosomes scattered along the length of the spindle (Figs. 9, 10, 29). After the chromosomes have reached the poles of the spindle, they may be separated from one another and remain scattered more or less widely in the cell. If the temperature is not too high (34°–35° C.) each separate chromosome will then swell up to form a separate vesicle, or if two or more chromosomes are in close contact they may form a single vesicle of larger size (Figs. 7, 9–12).

If the temperature is a little higher (37° C.) the chromosomes may stick together in an irregular mass and be drawn to the surface of the cell, probably by the complete contraction of the fibers which anchor the spindle to the peripheral layer of the protoplasm (Figs. 13, 14, 17). In the latter instance the chromosomes do not swell up and become vesicles, but remain permanently small and densely chromatic. Owing to some change, probably in the peripheral layer of each chromosome, caused by the high temperature, the chromosomes are unable to take up achromatin. Such chromosomes and the cells containing them never recover and never go further in development, although the cells do not immediately undergo degeneration and to all appearances remain alive for twenty-four hours or longer.

Of a piece with this scattering of chromosomes is the failure of all the chromosomal vesicles to unite into a single vesicle. All degrees of fusion of chromosomal vesicles may be found from those which remain wholly separate to those which are united into a single spherical vesicle. Other things being equal, the size of a nuclear vesicle varies according to the number of chromosomal vesicles which enter into it. In this way arise nuclear forms which have been called "fragmented nuclei," "multinuclear cells," "bipartite," "lobulated" and "elongated" nuclei (Figs. 1–12, 19–28), although it is evident in the case of *Crepidula* that these nuclear forms have not arisen by constriction or fragmentation of an originally single nucleus.

These partial nuclei formed by incomplete fusion of the chromosomal vesicles were first called "karyomeres" by Fol. That they are partial and not entire nuclei is shown by the fact first established by Boveri that in the next following mitosis each karyomere gives rise only to the same number of chromosomes as entered into it and not to the full number of chromosomes characteristic of the species; this fact I have repeatedly confirmed in my studies on *Crepidula*. And that such karyomeres are due to the failure of chromosomes to unite into one vesicle and not to the amitotic division of a single vesicle is shown by the following considerations:

1. They are most numerous in the telophase of division (Figs. 7–12) where the chromosomes are sometimes widely scattered

and where every chromosome may give rise to a separate chromosomal vesicle (Fig. 7). As the cell passes into the resting phase, these vesicles fuse together more or less completely, giving rise to vesicles of varying sizes. Other things being equal, the later the stage in the resting period the smaller the number of separate vesicles. If the separate vesicles were formed by division of an originally single vesicle, exactly the reverse would be the case.

2. The presence of centrospheres and spindle remnants in many cases shows conclusively that division has taken place by mitosis, and the position of these structures indicates the location of the mitotic figure (Figs. 8–12 *et seq.*).

3. The elongation of daughter nuclei in the position of the plate of daughter chromosomes of normal division figures indicates that such elongated nuclei are formed by the partial fusion of the chromosomes of the daughter plate (Figs. 4–6).

These considerations make it absolutely certain that these peculiar nuclear forms are due to a partial or incomplete union of chromosomes into a single nuclear vesicle in the final phase of mitosis; they represent modified mitosis and not amitosis. And it is practically certain that many of the cases of so-called amitosis described by several of the authors mentioned above are of this same character.

It is not easy to determine exactly the mechanism by which these modifications have been produced. As already mentioned they may be caused by abnormally high temperature (Figs. 1–12) or by hypertonic sea-water (Figs. 19–29). It is well known that when the daughter chromosomes approach the poles of the spindle they are normally closely crowded together and it seems probable that this is due to linin connections within the spindle or between the chromosomes. In the development of the daughter nucleus each chromosome absorbs achromatic material from the cytoplasm and becomes a vesicle with chromatic walls. The material thus absorbed is probably chiefly water though it doubtless contains dialyzable proteins and other substances which may be assimilated into the chromatin and linin of the nucleus. It is probable that there is a real membrane surrounding each chromosome (Conklin, 1902, 1912) and that the

absorption of surrounding substances by the chromosome takes place through this membrane by a process of dialysis. The nuclear vesicle is most nearly spherical in form when it is largest and when its contents are most fluid in character irregular or lobulated nuclei are usually smaller and the nuclear contents more dense (Figs. 1–6). Therefore the modifications of mitosis, which prevent the union of chromosomes into a single vesicle, act by modifying the walls of these vesicles so that they do not readily unite and so that they do not readily absorb fluid from the cytoplasm.

II. Amitotic or Mitotic Division of Nuclei Without Division of Cell Body and Subsequent Division of Such Binuclear or Polynuclear Cells.

In the cleavage of *Crepidula* a binucleate or multinucleate cell is invariably due, so far as I have observed, to a failure of chromosomal vesicles to unite into a single vesicle. In other cases, however, it is plain that elongated, constricted and bipartite nuclei have resulted from the constriction of a single nuclear vesicle. In addition to numerous cases which have been described by other investigators, I have myself studied such cases in the egg-follicle cells of *Gryllus* (Conklin, 1903), the liver cells of *Porcellio* (Conklin, 1897), as well as in some muscle cells and connective tissue cells. In none of these cases, however, is division of the nucleus followed by division of the cell body. Although every follicle cell of *Gryllus* and every liver cell of *Porcellio* shows the nucleus in some stage of amitosis, and although many of these cells contain two entirely separate nuclei, in no single instance have I ever seen a division of the cell body separating these nuclear halves.

Since these cases of amitosis occur in differentiated tissue cells it may be assumed that the nuclei are not active in the further differentiation of these cells; on the other hand their metabolic activity is great and the nuclei are undoubtedly concerned in this activity. It has been assumed that the division or lobulation of such nuclei favors metabolic activity by increasing the surface of the nuclei and bringing them into closer relation to all of the cytoplasm of the cell, and this is probably true. Espe-

cially in the case of elongated muscle cells the division of the nucleus and the distribution of the daughter nuclei along the length of the fiber must facilitate interaction between nucleus and cytoplasm, and the same is true, though perhaps to a smaller extent, in gland cells and egg-follicle cells.

Dahlgren and Kepner (1908) hold that the very numerous amitoses in the striated muscle cells of the embryo fish, *Catostomus*, may be followed in some instances by the division of the muscle cell. But since the plane of nuclear division is always transverse to the fiber, while the plane of cell division is always longitudinal, it could not be affirmed that the cell divisions in this case correspond to the nuclear divisions. But even if amitoses may be followed by division of the cell body in these cases, it must not be forgotten that all these cells are fully differentiated and according to the chromosome theory the nucleus has already performed its differentiating functions while its further function in the fully differentiated cell is probably purely trophic.

Many observations and experiments demonstrate that the nucleus is concerned in the two functions (1) of differentiation or regulation and (2) of metabolism; the work of Gruber (1886), R. Hertwig (1898), Heidenhain (1894), Henneguy (1896), Conklin (1902) *et al.* indicates that the chromosomes or basichromatin are particularly concerned with the former, the oxychromatin or achromatin with the latter. It is a significant fact that chromosomes divide only by mitosis and Boveri (1908) has shown that a complete set of chromosomes is necessary to normal differentiation. On the other hand oxychromatin and achromatin divide only by amitosis even in cases of mitotic division of the chromosomes. The significance of these facts seems to have been missed not only by those who maintain the equivalence of mitosis and amitosis, but also by Weismann and his followers who assumed that in embryonic differentiation there is a differential division of chromosomes and a "disintegration of the germ plasm" with segregation of particular factors in particular cells. For if individual chromosomes differ in hereditary potencies, every division by amitosis must be a differential one, while on the other hand every typical mitotic

division is non-differential so far as the chromosomes are concerned.

Since it is usually impossible to see outlines of individual chromosomal vesicles in the resting nucleus, it is not possible to determine whether the constrictions and lobulations of amitosis merely separate whole vesicles from one another. If they do the number of chromosomes arising from each of these vesicles in subsequent mitosis should be the same as in the case of karyomeres formed by the failure of vesicles to unite in the resting stage.

It is probable from the work of Boveri (1907) and of Macklin (1916) that when amitotic division of the nucleus is followed by mitosis, each nuclear vesicle gives rise to a fraction only of the normal number of chromosomes and that all the nuclear vesicles in a cell taken together give rise to no more than the normal number. Furthermore, the work of Boveri demonstrates that there is no return to the normal number of chromosomes when once a cell contains an abnormal number. Each nuclear vesicle produced by amitotic division is therefore a karyomere, in every way comparable to those produced by incomplete fusion of chromosomal vesicles; it is a fragment of a nucleus and not an entire nucleus, and this is equally true whether all the karyomeres lie within a single cell, as is usually the case, or whether in some rare instances they may be distributed to separate cells. In *Crepidula* it matters not how many karyomeres there are in a cell, if there are two and only two centrosomes all the chromosomes come together into a single plate and there is a normal division and distribution of each of these chromosomes to the daughter cells.

Therefore in considering the significance of amitosis it is of the utmost importance to know whether the constriction of the nucleus is followed by a division of the cell body; if it is not, amitosis is not a permanent nuclear division at all but merely a temporary separation of karyomeres which come together again into a unit structure at the next mitosis. It is a significant fact that in most instances amitotic division of the nucleus is not followed by division of the cell body.

In this connection it is worth while to compare with the

conditions just described those which obtain when mitosis is not followed by division of the cell-body. In a former paper (Conklin, 1912) I have described such cases at some length and need not here go into details. In brief, if the daughter nuclei and centrosomes lie so far apart in the undivided cell body that they do not interfere, in subsequent mitoses every one of these mitoses may be entirely normal and development may be typical except that no differentiation ever appears between the halves of the undivided cell. On the other hand, if the daughter centrosomes lie near together in the undivided cell body so that they interfere we get tripolar or tetrapolar figures with irregular distribution of chromosomes and usually with the formation of several karyomeres of varying sizes depending upon the number of chromosomes entering into them. Such multipolar mitoses in *Crepidula* are rarely followed by division of the cell body so that at every succeeding mitotic period the number of centrosomes and chromosomes in this undivided cell body are approximately doubled (Figs. 26–29). Of course such cells with abnormal numbers of chromosomes and centrosomes never develop normally. Normal differentiation depends upon the regular distribution into separate cells of daughter centrosomes and chromosomes as well as of different cytoplasmic substances.

III. Separation of Chromatin and Achromatin and Formation of Cytasters.

The behavior of the chromatic and achromatic parts of the nucleus in hypertonic and in hypotonic media throws a certain amount of light on the constitution of the normal nucleus and on the behavior of these nuclear constituents during normal mitosis. When resting nuclei are subjected to hypertonic solutions (*e. g.*, 2–4 per cent. NaCl in sea-water) the chromatic portion of the nucleus contracts into a small dense mass leaving the achromatic portion as large as ever (Figs. 32, 43, 48). It looks as if the chromatin had undergone complete "plasmolysis" whereas the volume of the achromatin had not been affected at all. The membrane or boundary of this achromatin remains full and unshrunken, which would presumably not be the case if this outline represented a real plasma membrane. The

shrinkage of the chromatin on the other hand probably indicates that it is surrounded by a plasma membrane, or more likely that each chromosome is so surrounded.

When resting nuclei are subjected to hypotonic solutions the entire nucleus becomes slightly swollen and less deeply chromatic, which indicates that the chromatic parts of the nuclei take up water, probably through the chromatic nuclear membrane or the chromosomal membranes.

The achromatic membrane, or rather boundary, is regularly spherical in resting stages but during mitosis it disappears or else becomes so indefinite and irregular in outline that it is difficult to recognize. However the achromatic substance of the nucleus together with some of the denser portion of the cytoplasm constitutes the amphiaster with its nuclear spindle and astral radiations. In hypertonic solutions the amphiaster is sharply set off from the surrounding cytoplasm (Figs. 35–36), due as I believe in the main to the condensation of its substance and the elimination from it of the more fluid cytoplasm. In this process of condensation the astral radiations are largely drawn into the central part of the figure but portions of these radiations may become isolated from the amphiaster and thus form independent condensation centers. These have a radiating structure and are typical cytasters, but unlike those described by Wilson they do not in *Crepidula* divide nor form the poles of true mitotic figures. My observations on the origin and nature of these cytasters (Conklin, 1912) entirely agree with those of Konopacki (1911) and in the main with the observations of Mead (1898) and Morgan (1899).

Cytasters appear best developed during periods of mitosis when the achromatin is distributed in the astral radiations (Figs. 33–35) but they are also abundant in eggs after the maturation divisions and before the first cleavage (Figs. 31, 32) and in such cases one can frequently see that they lie along the radiations of the maturation aster (Fig. 31).

During prolonged resting periods, especially when the eggs are in strong salt solutions, cytasters are replaced by faintly staining vesicles (Figs. 37–42) which appear to contain achromatic nuclear material. These vesicles are surrounded by a delicate achromatic

membrane and they resemble R. Hertwig's "nuclei without chromatin." They are found chiefly in the position of the previous spindle remnants and along the lines of astral radiations. Usually the largest of these achromatic vesicles are in close proximity to the dense mass of chromatin, which in these cases does not become vesiculated. In some instances there is a single elongated achromatic vesicle in each daughter cell which occupies the position of the interzonal fibers of the spindle (Figs. 43–48) and which may inclose the dense mass of chromosomes at the spindle ends. Such conditions give the appearance of an amitotic division of the nuclear vesicle, but the presence of centrosomes, mid-bodies and in some instances of spindle fibers and astral radiations as well as of chromosomal plates (Figs. 46, 47) clearly shows that these divisions are true mitoses in which the chromosomes have been prevented from absorbing achromatin while the latter has formed a definite boundary or membrane separating it from the cytoplasm.

Just as the size of a central aster is reduced by the presence of numerous cytasters or parasitic asters which surround it, so the size of the chromatic nuclear vesicle is inversely proportional to the volume of the achromatic vesicles in the cell. It seems practically certain that the chromosomal vesicles and consequently the entire chromatic portion of the nucleus grow by the absorption of this achromatic substance. When nuclei are large they contain much achromatic substance and at the same time there are no cytasters or achromatic vesicles in their vicinity; when they are small and densely chromatic there may be cytasters in the cell during the periods of mitosis, or achromatic vesicles during resting periods. "The cytasters are therefore, in my opinion, isolated portions of archiplasm (achromatin plus spongioplasm) derived in large part from escaped achromatin, which take the aster form during mitosis and the vesicular form during resting periods" (Conklin, 1912, p. 543).

IV. Persistence of Nuclear Membranes and Formation of Chromatic Connections Between Daughter Nuclei.

It has generally been assumed that one of the strongest evidences that amitosis had occurred in any given case was to be

found in the incomplete separation of daughter nuclei or in chromatic connections between them. Thus Gurwitsch (1905) has figured and described the division of a blastomere of a centrifuged *Triton* egg in which two nuclei, connected by a chromatic thread, are dividing by mitosis. The chromatic connection is taken as proof positive that the nuclei had divided by amitosis and Godlewski (1909) in a general review of this subject, after dismissing as doubtful many other cases in which amitosis had been reported as occurring in normal development, falls back upon this case described by Gurwitsch as one of the strongest evidences in favor of the view that amitosis may occur in normally differentiating cells.

But chromatic connections between nuclei are not to be taken as positive evidence that those nuclei have divided by amitosis, for these connections may be the result of incomplete or atypical mitoses. Anything which retards or prevents the separation of daughter chromosomes may lead to the scattering of chromosomes along the spindle or to their elongation into threads and consequently to the formation of chromatic connections between daughter nuclei. Häcker found such connections in etherized eggs of *Cyclops* and such connections are present also in *Crepidula* eggs subjected to high temperatures (Figs. 6, 8), to hypertonic sea-water (Fig. 29), and to hypotonic sea-water (Figs. 49–60). It is especially in the last named experiments that chromatic connections between daughter nuclei are most frequently seen and they merit a detailed description.

The eggs shown in Figs. 49–54 were placed for one hour in sea-water diluted with two volumes of fresh water and were then returned to normal sea-water for four hours before being fixed; those shown in Figs. 55–60 were placed for two hours in sea-water diluted with one volume of fresh water and were then left in normal sea-water for fourteen hours. In all of these cases the centrosomes divided normally and approximately normal spindles were formed but the separation of chromosomes and the formation of daughter nuclei were atypical. In Figs. 9, 29, 49 and 50 the scattering of chromosomes is shown in some of the spindles but more notable than this is the stretching of chromosomes into long threads some of which run from one

pole of the spindle to the other. When chromosomes are merely scattered throughout the cell or along the spindle they usually give rise to chromosomal vesicles wherever they lie, as is shown in Figs. 7, 16, 26, etc., but when in addition they are stretched into elongated threads chromatic connections are left between daughter nuclei.

In diluted sea-water the chromosomes show a tendency to stick together into masses and to stretch out into long threads instead of dividing and moving to the two poles of the spindle. This is probably due to the fact that the linin basis of the chromosomes is modified so that the latter do not preserve their usual shapes and do not separate normally in division. The nuclear membrane also frequently remains chromatic and in such cases may persist throughout mitosis (Figs. 49–53, 55, 59, etc.). Evidently some of the chromatin which usually enters into the formation of the chromosomes is left in these cases at the periphery of the nucleus. Since the nucleus is composed of chromosomal vesicles more or less completely united this result might conceivably be due to the swelling and bursting of some of these vesicles at the nuclear periphery.

When mitosis is halted in the prophase of the third cleavage the centrosomes separate and a spindle is formed in the usual manner but the nuclear membrane persists and the entire nucleus becomes pear-shaped (Fig. 49, cell D), or unequally constricted (Figs. 51, 52), the smaller portion corresponding to the micromeres containing almost all of the deeply staining chromatin in the form of chromatic threads, while the larger portion belonging to the macromeres contains faintly staining threads and granules. These two portions of the constricted nuclei are approximately proportional in size to the nuclei of the normal micromeres and macromeres, although in cell D, Fig. 49, and cells C and D, Fig. 52, the division wall between the micromeres and macromeres has not formed. The fact that these two portions of the constricted nuclei are proportional in size to the cells to which they belong even when the division wall between those cells has not formed is difficult to explain. Generally the size of a nucleus is proportional to the volume of cytoplasm in which it lies (Conklin, 1912) because the chromosomal vesicles

absorb substance from the cytoplasm in their growth, but in this case the entire nucleus has undergone constriction and the cell body has not.

The aggregation of chromatin on the side of the nuclear vesicle on which the spindle lies causes it to collect on the animal pole side of the nucleus in Figs. 49–51 and on the side away from the animal pole in cell D, Fig. 54. In every instance the chromatin collects at that pole of the nucleus which is next to the centrosome or spindle; this is a general phenomenon which has been remarked by R. Hertwig in *Actinospherium*, by Calkins in *Noctiluca*, Conklin in *Crepidula*, etc. In this connection one recalls that in many protozoa the nuclear membrane persists throughout mitosis the spindle being within the nuclear vesicle. The chromosomes divide and separate as in typical mitosis but the nuclear membrane and vesicle constrict as in amitosis. In the ciliata the micronucleus divides by mitosis, the macronucleus by amitosis. In metazoa also the chromosomes alone divide by mitosis, or the splitting of the chromatic thread, while the division of all other nuclear constituents is a mass division.

The peculiar form of nuclear division which is caused by the stretching out of the chromosomes and the persistence of the nuclear membrane superficially resembles amitosis but is really a modified form of mitosis. Nuclei which have divided in this peculiar manner go on dividing by mitosis when they are returned to normal sea-water. Thus cells A and C, Figs. 49 and 50, cells A and B, Figs. 52 and 53, and all the macromeres in Fig. 54 are shown dividing by mitosis. These are typical mitotic figures though the number and arrangement of chromosomes may be atypical. In all of these cases the dividing nucleus of the macromere is connected with the nucleus of the micromere by a chromatic strand and it is particularly noticeable that this strand always runs to the plate of chromosomes in the macromeres and usually to the outer side of this plate. Since the original chromatic connection united the nuclei by their distal poles (away from the centrosome) the fact that when these nuclei divide the connection runs to the proximal pole (toward the centrosome) indicates that the chromatin changes position within the nuclear vesicle, being drawn to the proximal pole of the nucleus (Fig. 54, *D*).

Again the way in which the chromatic connection between nuclei unites with the chromosome plate (Figs. 53, 54) shows that this connection is actually composed of chromosomal substance though it has been so modified that it does not give rise to separate chromosomes nor does it show any tendency to divide or split, as normal chromosomes do, into daughter chromosomes. Although it is not possible to count the number of chromosomes in these plates it is evident that it varies in different cases and that in general it is less than normal, which is what would be expected if the chromatic connections represent a number of spun-out chromosomes. Furthermore the fact that these connections do not swell up to form vesicles as normal chromosomes do, indicates that the chromosomal substance of which it is composed has undergone some significant change.

Still later divisions of the cells connected by these chromatic strands are generally abnormal, as is shown in Figs. 55–60. The eggs shown in these figures were subjected to diluted sea-water during the third cleavage and were then returned to normal sea-water where some of the cells have undergone the fourth, fifth and sixth cleavages. In many cases the chromosomes at each of these cleavages have been stretched out into chromatic connections between daughter nuclei and since all of these persist a complicated network of such connections is present between nuclei of successive generations (Figs. 55, 56, 58, 60); at the same time the number of chromosomes in a plate is in many instances greatly reduced (Fig. 55).

The same types of modifications produced by diluted sea-water persist after the eggs have been returned to normal sea-water and in all of these cleavages, if one saw only the end result, the chromatic connections would seem to indicate that the nuclei had divided by amitosis. However a study of various stages in this process shows conclusively that this is not the case but that all of these divisions are modified forms of mitosis.

V. Conclusions.

1. The modern revival of interest in amitosis is due to a reaction against the chromosome theory. If nuclear and cell divisions ever take place by amitosis in normally developing sex

cells and embryonic cells it would deal a fatal blow to that theory. The occurrence of amitosis in fully differentiated tissue cells or in cells which do not undergo division would not affect the chromosome theory.

2. When direct division of the nucleus occurs it is rarely if ever accompanied by division of the cell body. The individual nuclear vesicles or karyomeres are not whole nuclei but fragments of a nucleus and when the cell actually divides these karyomeres combined form the typical number of chromosomes which unite into a single spindle and divide in the typical manner, as recently shown by Macklin.

3. Many apparent cases of amitosis are merely modified mitoses of which the following forms are described in this paper:

(*a*) The scattering of chromosomes and their failure to unite into a single nuclear vesicle.

(*b*) Mitotic division of the nucleus without division of the cell body and the consequent formation of binucleate or polynucleate cell.

(*c*) The failure of daughter chromosomes to pull apart in the spindle and the consequent formation of chromatic connections between daughter nuclei.

(*d*) The persistence of the nuclear membrane with division of the chromosomes by mitosis and of the nuclear vesicle by constriction.

4. There is not a single wholly conclusive case in which amitosis has been shown to occur in the division of normally differentiating cells. Therefore the attempts to disprove the chromosome theory in this way have failed.

5. Mitosis and amitosis are fundamentally unlike. Mitosis is the one and only method of bringing about equal division and distribution of the chromatic material of the nucleus. Amitosis is not a genuine divisional phenomenon at all but merely a means of increasing the nuclear surface and of distributing nuclear material throughout the cell, comparable to nuclear lobulation, fragmentation or distribution. These two processes are not equivalent or even comparable nor may one of them be converted into the other.

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Description of Figures.

Figs. 1–60 represent whole eggs of *Crepidula plana* which had been fixed, stained and mounted. All figures were drawn with camera lucida under Zeiss apochromatic objective 3 mm. Hom. Im., Comps. ocular 4. As drawn they represent a magnification of 333 diameters; in the process of reproduction they have been reduced about one third.

Figs. 1–18 represent eggs which had been subjected to increased temperature.

Explanation of Plate I.

Fig. 1. No. 1174(2). 37°, ¼ hr., during second maturation; then kept at room temperature (ca. 27°) for 3 hrs. The first polar body is shaded by transverse lines; the sperm nucleus is shown at the left; the other nuclei are karyomeres formed by the scattering of chromosomes of the second maturation mitosis.

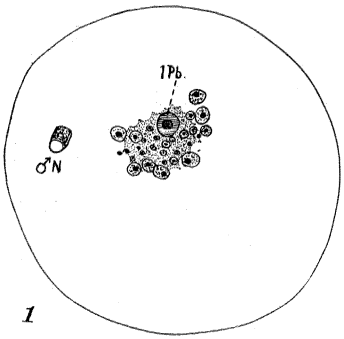
Fig. 2. No. 1175(2). 33°, ¼ hr., during first cleavage; room temperature (ca. 27°). 3 hrs. The imperfect fusion of the karyomeres (gonomeres?) gives an appearance of amitosis to each of the nuclei.

Fig. 3. No. 1176(2). 34°–35°, ¼ hr., during second cleavage; room temperature (ca. 23°) 3 hrs. Karyomeres formed by scattering of chromosomes of second cleavage.

Fig. 4. No. 1173(2). 37°, ¼ hr., beginning of third cleavage; room temperature (ca. 27°) 3 hrs. Many karyomeres formed by scattering of chromosomes of third cleavage. Traces of centrospheres are shown in the cells.

Fig. 5. No. 1176(2). 34°–35°, ¼ hr., during third cleavage; room temperature (ca. 23°) 3 hrs. Karyomeres formed by scattering of chromosomes of third cleavage. In one quadrant a micromere was formed, in the other quadrants cell division was suppressed. Traces of centrospheres as in preceding.

Fig. 6. No. 1173(2). 37°, ¼ hr., during anaphase of third cleavage; room temperature (ca. 27°) 3 hrs. In two quadrants micromeres have formed. Daughter nuclei are elongated in the greater dimensions of the chromosome plates, the latter having fused together into irregular masses.



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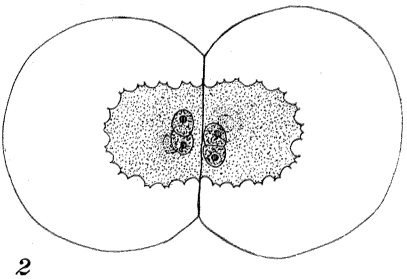

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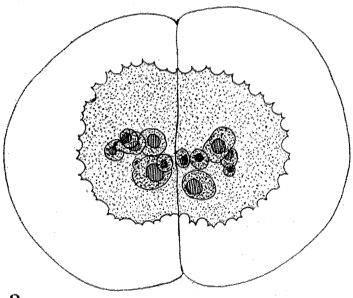

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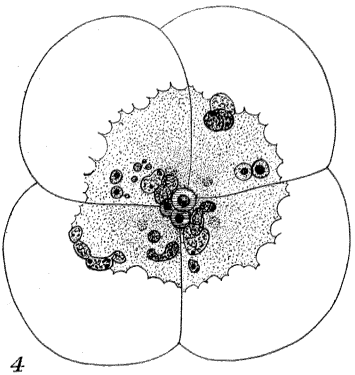

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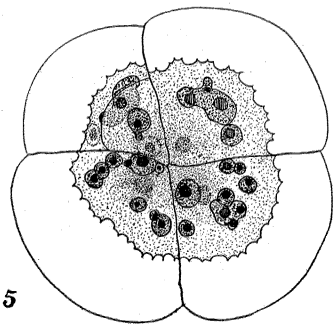

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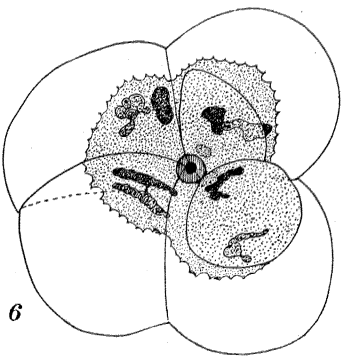

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Explanation of Plate II.

Fig. 7. No. 960. Ca. 35°, 4 hrs., during third cleavage. The chromosomes have scattered and formed numerous chromosomal vesicles.

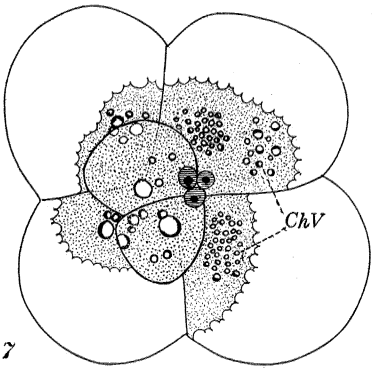
Fig. 8. No. 1176(2). 34°–35°, ¼ hr., during anaphase of third cleavage; room temperature (ca. 23°) 3 hrs. Many nuclei are lobed or irregular; nuclei are most abnormal in the quadrant in which a micromere did not form, and in which mitosis was not so far advanced at the time of the experiment, as in the other quadrants.

Fig. 9. No. 1176(1). 34°–35°, ¼ hr., during fourth cleavage of macromeres. In three quadrants the chromosomes are scattered in the spindles, in the fourth are chromosomal vesicles. The nuclei and centrosomes in the first set of micromeres are in a resting stage and are absolutely normal.

Fig. 10. No. 1176(1). 34°–35°, ¼ hr., during fourth cleavage. Chromosomal vesicles are present in one of the macromeres, and chromosomes are more or less scattered in all micromeres of the first set.

Fig. 11. No. 1176(2). 34°–35°, ¼ hr., during fourth cleavage; room temperature (23°) 3 hrs. The nuclei formed after the division of the first set of micromeres are lobed and irregular; the scattered chromosomes in the macromeres have formed many karyomeres. This figure shows an egg like Fig. 9 after being kept three hours at normal temperature.

Fig. 12. Same slide as preceding. Many lobulated nuclei and karyomeres in the cells derived from the first set of micromeres; nuclei in second set of micromeres and in macromeres are nearly normal. This figure shows an egg like Fig. 10, after being kept three hours at normal temperature.



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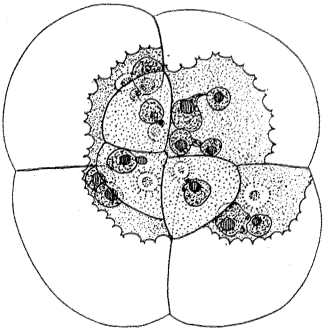

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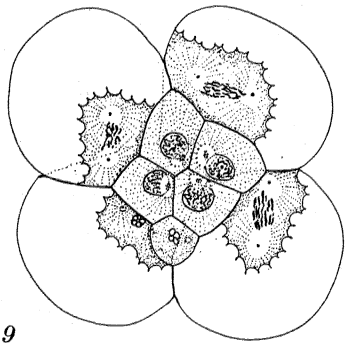

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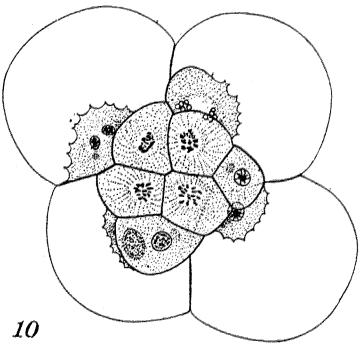

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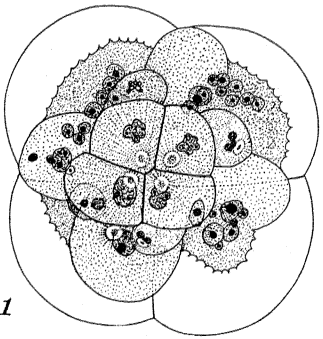

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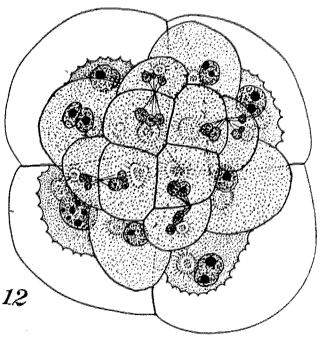

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Explanation of Plate III.

Figs. 13–18 were subjected to temperature so high (37°), or for so long a period (35° for four hours) that further cell division was stopped in almost all cases, though the protoplasm remained transparent and apparently alive.

Fig. 13. No. 1171(2). 37°, ½ hr. during first cleavage; room temperature (ca. 25°) 15 hrs. Astral areas are large and distinct; chromosomes are widely scattered or clumped in two principal masses outside the astral areas and at the surface of the egg; chromosomes do not become vesicular.

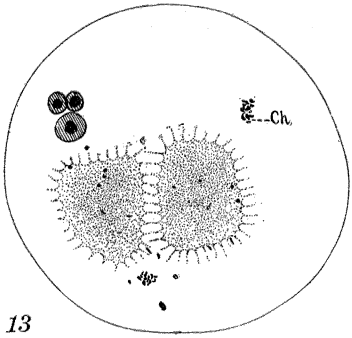
Fig. 14. Same slide as preceding; subjected to heat during second cleavage, and showing results similar to preceding.

Fig. 15. No. 960. Ca. 35°, 4 hrs., during resting 2-cell stage. The plasma is much contracted and vacuolated and the chromatin of the nuclei is in the form of hollow spheres, which look like chromosomal vesicles.

Fig. 16. Same slide as preceding; heated during second cleavage mitosis; plasma shrunken and chromosomes in the form of vesicles.

Fig. 17. No. 1171(2). 37°, ½ hr., during fourth cleavage; room temperature (ca. 25°) 15 hrs. Chromosomes in the macromeres are clumped together at the surface of the egg, and are outside the plasma areas. There is a triaster in one of the micromeres, while the others appear normal.

Fig. 18. Same slide as preceding; 24-cell stage. All centrospheres instead of being at the surface, as in normal eggs, are at the deeper ends of the cells next to the spacious segmentation cavity.



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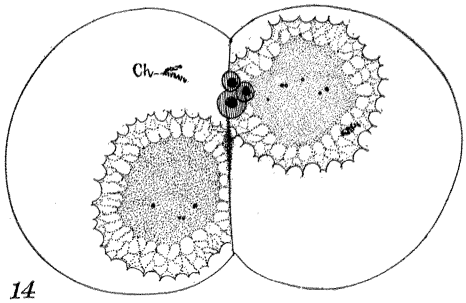

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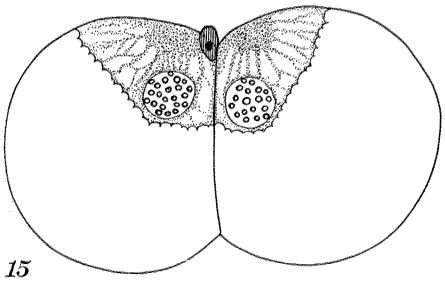

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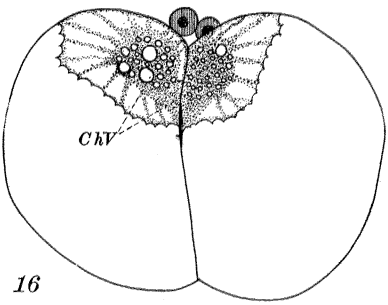

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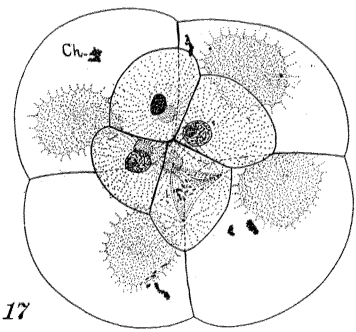

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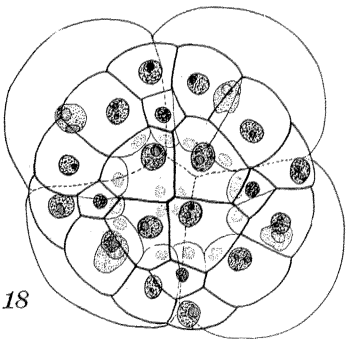

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Explanation of Plate IV.

Figs. 19–48 (with exception of Fig. 30) represent eggs which had been subjected to hypertonic solutions.

Fig. 19. No. 822. 2 per cent. NaCl, 16 hrs., normal sea-water 8 hrs. Numerous karyomeres were formed after maturation and before the first cleavage, presumably during the first cleavage mitosis.

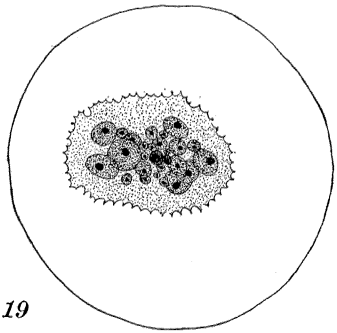
Fig. 20. Same slide as preceding. Karyomeres formed at the close of the first cleavage by chromosomes failing to unite. Centrospheres (*s*) unusually large.

Fig. 21. No. 837. $\frac{3}{4}$ per cent. KCl 9 hrs.; normal sea-water 35 hrs. Karyomeres irregular in size, shape and distribution.

Fig. 22. Same slide as preceding, with similar karyomeres.

Fig. 23. No. 822. 2 per cent. NaCl 16 hrs.; normal sea-water 8 hrs. Second cleavage abnormal, with karyomeres in two of the cells.

Fig. 24. No. 814. 2 per cent. NaCl 1 hr.; normal sea-water 17 hrs. Third and fourth cleavages abnormal, with karyomeres in most of the cells.



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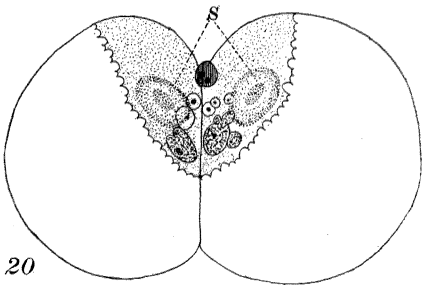

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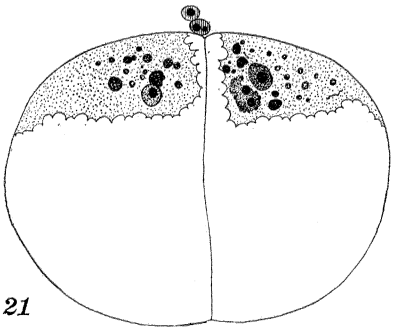

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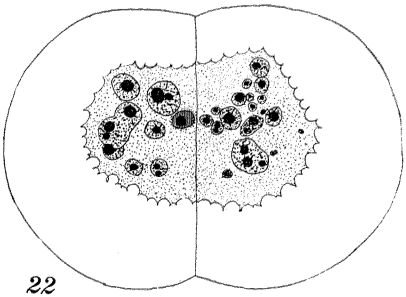

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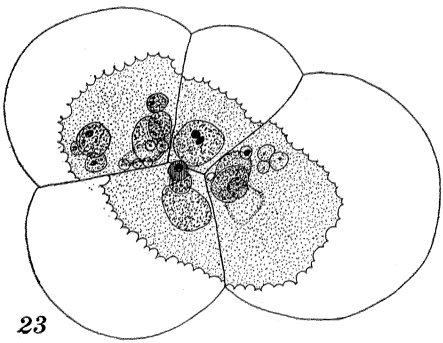

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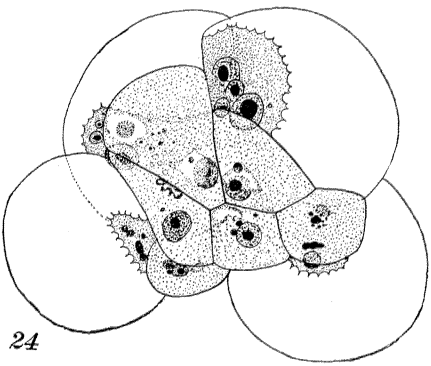

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EXPLANATION OF PLATE V.

FIG. 25. No. 927. 2 per cent. NaCl 16 hrs., at close of third cleavage; normal sea-water 24 hrs. Numerous karyomeres in the cells.

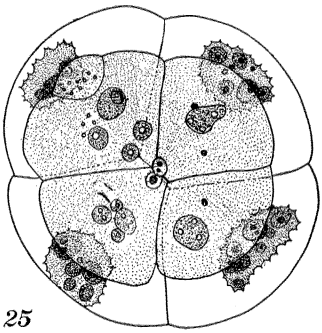
FIG. 26. No. 867. 8 per cent. $MgCl_2$ $\frac{3}{4}$ hrs., during third cleavage; normal sea-water $6\frac{1}{2}$ hrs. Numerous karyomeres in resting cells; in dividing cells, polyasters and scattered chromosomes.

FIG. 27. No. 972, same slide as Fig. 25. Karyomeres in all resting cells; polyasters and scattered chromosomes in dividing ones.

FIG. 28. No. 971. 2 per cent. NaCl 16 hrs., during third cleavage; normal sea-water 12 hrs. Similar to preceding.

FIG. 29. No. 828. 1 per cent. NaCl 2 hrs., during third cleavage; normal sea-water $6\frac{1}{2}$ hrs. Polyasters and chromatic connections between daughter nuclei.

FIG. 30. No. 956. Sea-water diluted with equal parts of fresh water 2 hrs.; normal sea-water 36 hrs. The nuclei in yolk-containing cells show many karyomeres.



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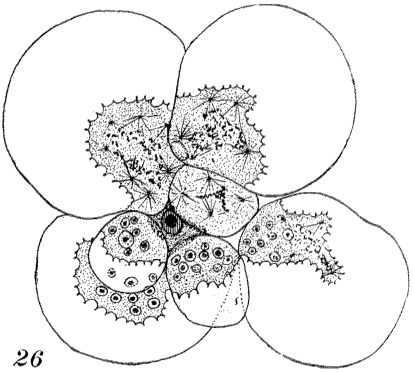

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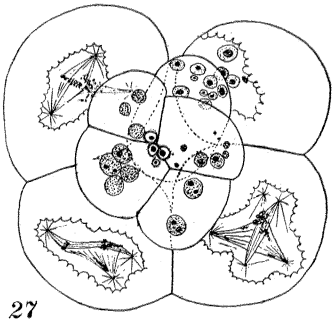

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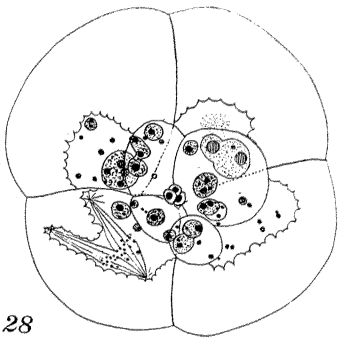

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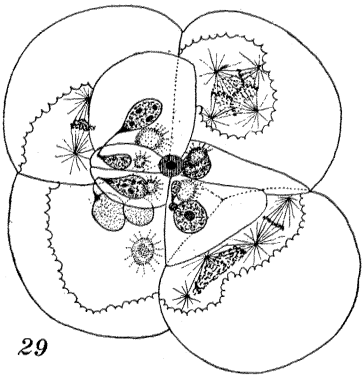

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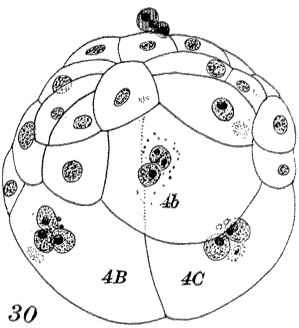


30

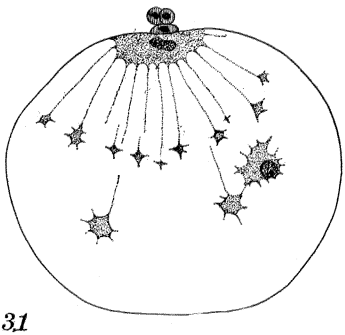
EDWIN G. CONKLIN.

Explanation of Plate VI.

Fig. 31. Normal egg showing cytasters sometimes present in eggs at the stage between the close of the maturation divisions and the beginning of the first cleavage. These cytasters are local aggregations of plasma along the lines of astral radiations.

Figs. 32, 34, 35. Eggs from Exp. No. 805. 2 per cent. NaCl 4 hrs. Numerous cytasters are shown in different cell stages and division phases; in every case these cytasters are local aggregations of plasma along the lines of astral radiations, the remaining plasma being gathered around the nuclei or spindles.

Figs. 33, 36. No. 998(3). 1 per cent. NaCl 5 hrs. The plasma is concentrated around the spindles, only small portions being left along the astral radiations in the 2-cell stage and none in the 4-cell stage. No cytasters are present after the 2-cell stage.



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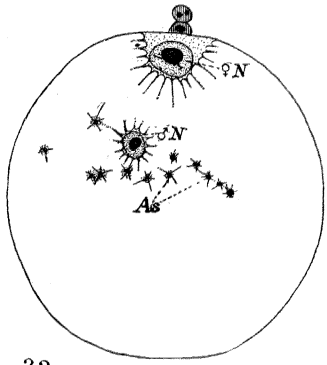

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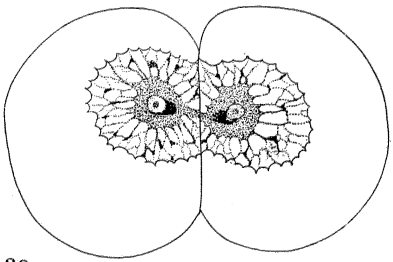

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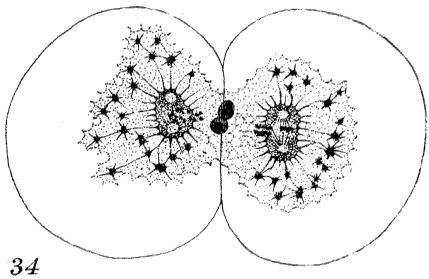

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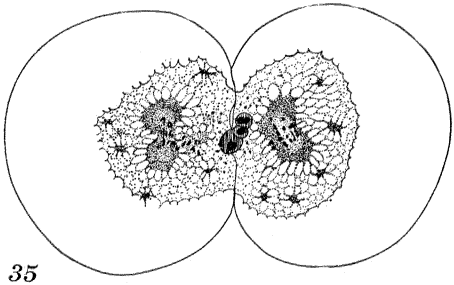

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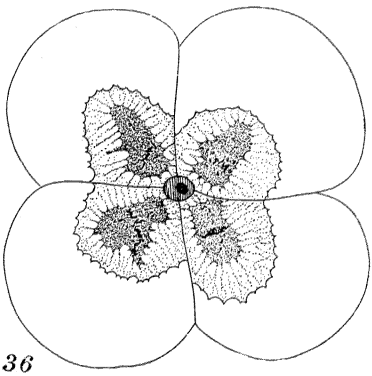
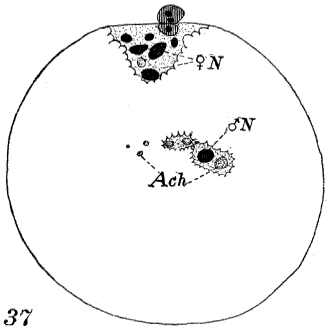


36

EDWIN G. CONKLIN.

Explanation of Plate VII.

Figs. 37–42. Various eggs from one experiment, viz., No. 809. 2 per cent. NaCl 15½ hrs. All figures show the chromatin in a densely chromatic mass and in all cases except Fig. 37, where the egg nucleus consists of several karyomeres, all the chromatin of each nucleus is aggregated into a single mass. On the other hand the achromatin consists of vesicles of different sizes which are more or less widely scattered, though a large achromatic vesicle is usually found adjoining a chromatic mass. This achromatin has not been squeezed out of the chromatic mass by the action of the salt solution, but the chromosomes of the daughter nuclei have been prevented from absorbing achromatin by the action of the salt solution.



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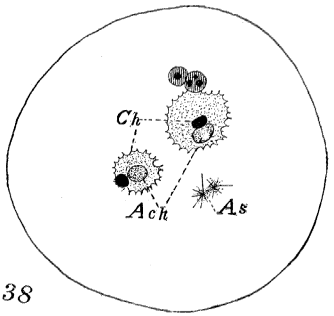

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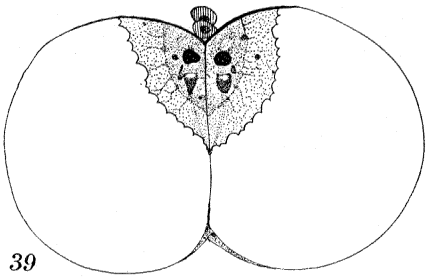

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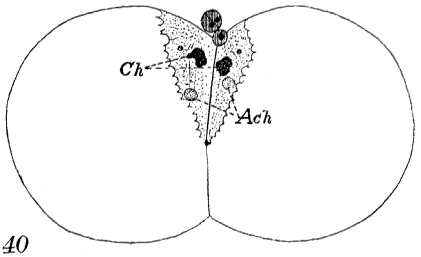

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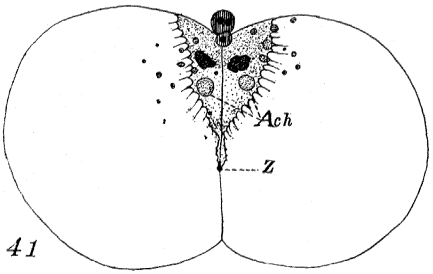

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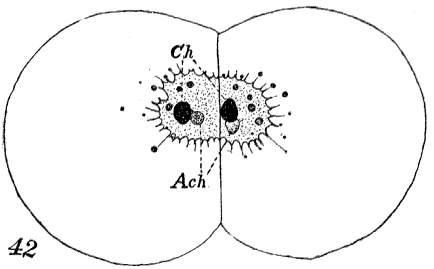
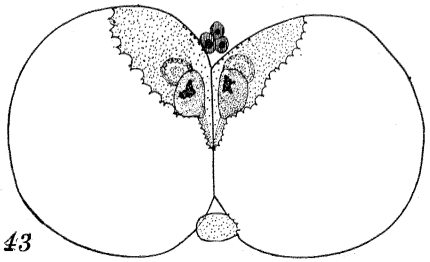


42

Explanation of Plate VIII.

Figs. 43–48 are different eggs from the same experiment, viz., No. 810. 3 per cent. NaCl 15½ hrs. All the eggs were in the anaphase or telophase of the second cleavage at the time they were placed in the salt solution, and in all cases the chromosomes have remained in the form of a dense chromatic plate or mass which has not become vesicular. In Fig. 45, the spindle remnants between the chromosomal plates has become vesicular; in Figs. 46 and 47, the spindle area has become an elongated achromatic vesicle, within which lies the dense chromatic mass; in Figs. 43, 44, 48 the achromatic vesicle has become more nearly spherical in outline, while the chromatic mass is not quite so dense as in earlier stages. These figures show that when the chromosomes are prevented from absorbing achromatin and becoming vesicular nuclei, the achromatin of the spindle region may become a vesicle by the formation of a delicate achromatic membrane around itself.



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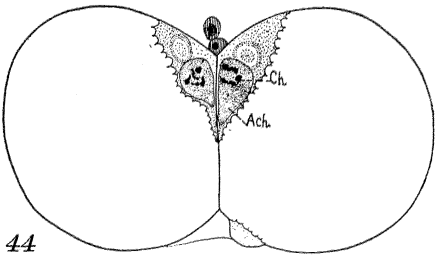

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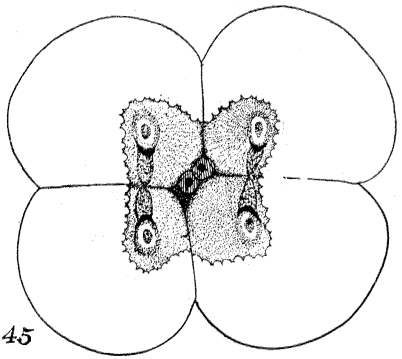

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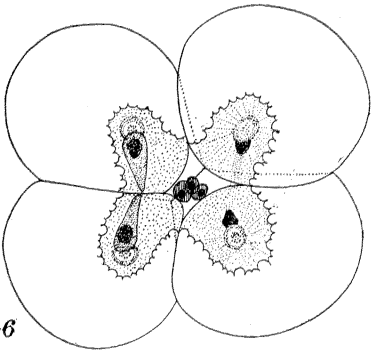

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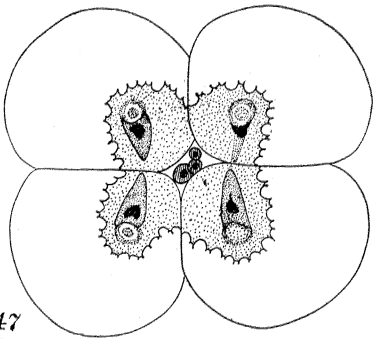

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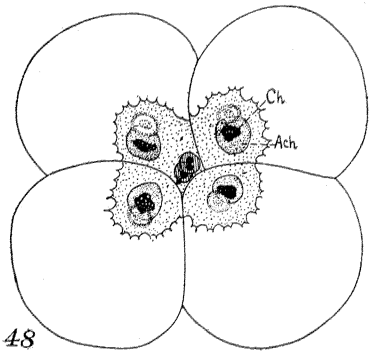


48

EDWIN G. CONKLIN.

Explanation of Plate IX.

Figs. 49–60 represent eggs which had been subjected to diluted sea-water.

Figs. 49–54. No. 859. Sea-water 1 part, fresh water 2 parts, 1 hr.; normal sea-water 4 hrs. Eggs were treated with this diluted sea-water during the third and fourth cleavages, thus causing a scattering or stretching out of chromosomes along the spindle and the formation of chromatic connections between daughter nuclei. In cell D, Fig. 49, the division was stopped in the prophase, the nucleus being pear-shaped with the chromatin chiefly in the narrow upper end of the pear. It is significant that the long axis of the pear is in the direction of the spindle axis and that if the constriction were to separate the neck from the body of the pear, the daughter nuclei thus formed would be of approximately the same size as in normal eggs.

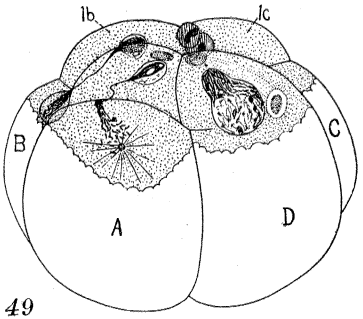
Fig. 51 shows more advanced stages of a similar process in which the chromatin is chiefly in the smaller, upper nuclei, the achromatin in the larger, lower ones.

Figs. 49 and 50 show certain nuclei in which the nuclear membrane remains intact though the chromosomes are arranged along a line or spindle connecting the two centrosomes.

Figs. 52–54. Eggs in which the third cleavage took place by a modified form of mitosis which left chromatic connections between daughter nuclei, and yet typical spindles for the fourth cleavage are present in some of the macromeres.

Fig. 52. In all four quadrants of this egg nuclear division at the third cleavage took place by modified mitosis, the daughter nuclei remaining connected by chromatic threads; in only two quadrants were micromeres formed, and the macromeres of these quadrants are now dividing by mitosis.

Figs. 53, 54. All daughter nuclei are connected by chromatic threads. Chromatin aggregates on the side of the nucleus next the centrosome (Fig. 54, *D*) and the chromatic connections between daughter nuclei run to the outer sides of the nuclei and spindles in the macromeres; the latter may be approximately normal, though the spindles may be out of proper position and the chromosomes more or less scattered.



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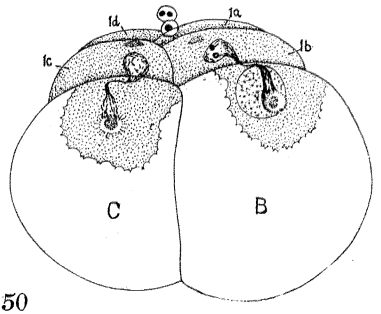

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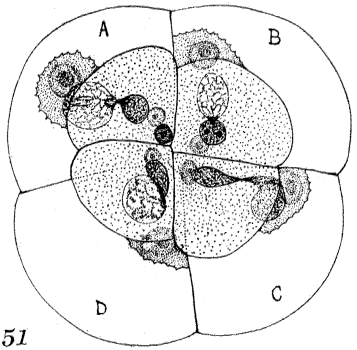

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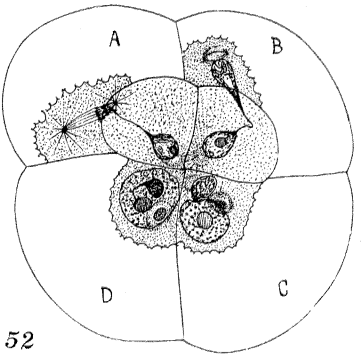

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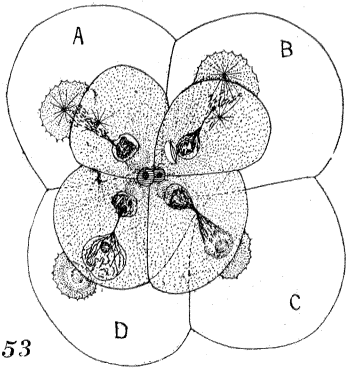

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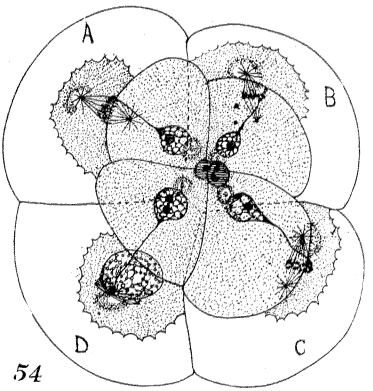
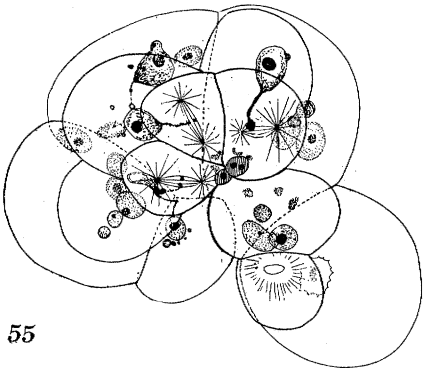


54

Explanation of Plate X.

Figs. 55–60. All from one experiment, No. 858. Eggs in 2–4-cell stage were left in sea-water diluted with equal parts of fresh water for two hours; then in normal sea-water fourteen hours. Remains of chromatic connections which were formed during the third cleavage are seen in Figs. 55, 56, 60. Other chromatic connections which were formed in later cleavages after the eggs had been returned to normal sea-water are shown in Figs. 57–60.



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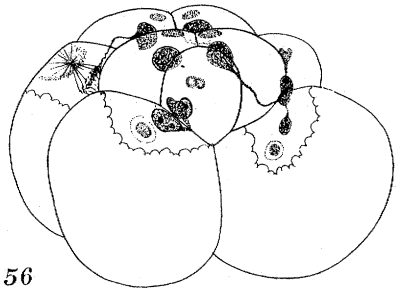

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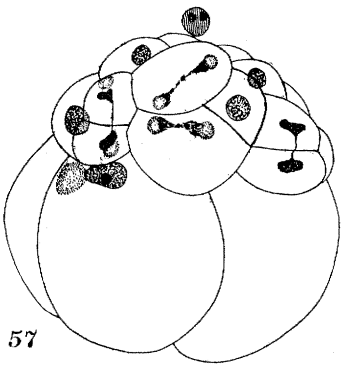

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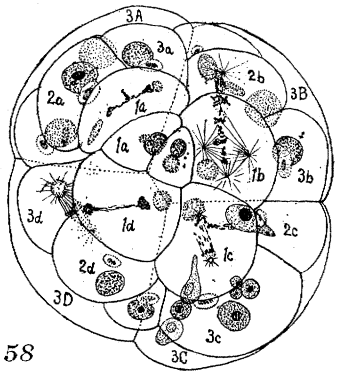

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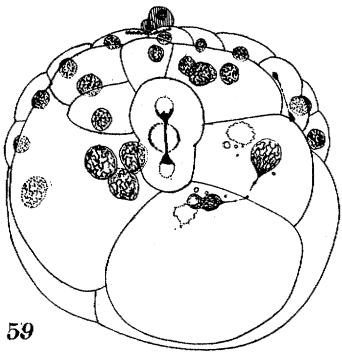

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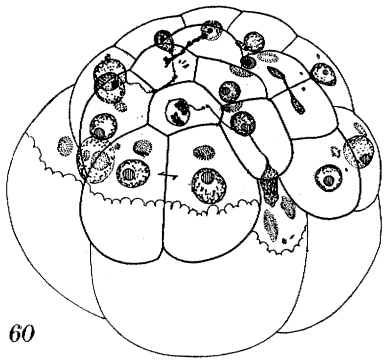

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